Transcriptome Analysis of the Blueberry-Mummy Berry Pathosystem

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Abstract
Mummy berry disease of blueberry, casual agent Monilinia vaccinii-corymbosi, has two distinct phases- a blight stage of young foliage and flowers and a flower infection stage that leads to mummified fruit (pseudosclerotia). The flower infection stage requires conidia to germinate on the stigma and grow down the stylar canal into the ovary. The cultivar Berkeley is very susceptible to the mummy berry fruit infection stage while the cultivar Bluejay is resistant. We reasoned that the resistance/susceptibility reaction might be ‘expressed’ in the style. Open flowers of both cultivars were pollinated with ‘Bluecrop’ pollen and inoculated with mummy berry conidia. Total RNA was isolated from the styles two days post-pollination/inoculation. RNASEq libraries were prepared and sequenced using the Illumina HiSeqII platform. The resulting sequences were then quality- and adapter-trimmed with open-source software ‘cutadapt.’ Sequences were assembled using Velvet. This produced 37,354 contigs for ‘Bluejay and 36,202 for ‘Berkeley’ of at least 200 nucleotides in length. The sequences were then BLASTed against the NCBI’s nucleotide database (blastn), which yielded 33,184,648 and 21,143,803 ‘hits’ for the two varieties, respectively. The hits were then mapped to their respective taxonomies. The data generated will help determine the key genes involved in the plant-pathogen interaction.

Keywords
Monilinia vaccinii-corymbosi, RNASEq, flower infection

Introduction
The plant-pathogenic fungus, Monilinia vaccinii-corymbosi, infects blueberry flowers via the gynoecial (stigma-style-ovary) pathway, causing mummy berry disease, an economically important disease of wild and cultivated blueberries. Many plant-pathogenic fungi and bacteria are capable of infecting the flowers of their host plants, causing diseases that are economically important because of their direct impact on seed and/or fruit set (Ngugi and Scherm, 2006). Although most flower pathogens are non-specialists, i.e., they are capable of infecting other plant organs such as leaves, other flower pathogens are more specialized, that is, they are capable of infecting the flower without blighting it, in some cases utilizing the pollination pathway (stigma-style-ovary) to gain entry into the ovary. These same pathogens typically also require flower infection to complete their life cycle, e.g., to accomplish formation of a sclerotium or pseudosclerotium. The
pseudosclerotium germinates in the spring and forms ascospores that initiate the blight phase of the fungus on leaves and sometimes flowers. Conidia form on the blighted tissue and are transferred, usually by bees, to the stigmas of open flowers where they germinate and begin the flower infection part of the life cycle.

We presume that host resistance to either part of the life cycle (blight or flower infection) will reduce disease pressure due to limited completion of the life cycle (and thereby inoculum). Many blueberry cultivars have been tested for resistance to one or both phases of mummy berry (Ehlenfeldt et al., 1996; Ehlenfeldt et al., 2010; Stretch and Ehlenfeldt, 2000). It has been noted that in cultivars exhibiting resistance to the flower infection stage that a component of resistance is expressed during growth in the gynoecial pathway (Lehman et al., 2007). It is this stage that was targeted in this study.

The main objective of this project is to determine the key genes associated with the blueberry-mummy berry plant-pathogen interaction. Our specific objectives are to: 1) determine the transcriptome of blueberry flower styles during invasion by the mummy berry fungus and establish which gene transcripts (or transcript levels) are differentially affected by pathogen ingress in styles of resistant vs. susceptible blueberry cultivars and 2) determine what genes are associated with resistance and to use those genes to develop markers for resistance to be used in MAS (marker assisted selection).

**Materials and Methods**

**Plant material:** Blueberry cultivars used were ‘Bluejay’ (resistant), ‘Berkeley’ (susceptible) and ‘Bluecrop’ (pollenizer). All plants were 3-5 year old potted plants.

**Inoculum:** *Monilinia vaccinii-corymbosi* was isolated from infected blueberry at the P.E. Marucci Center for Blueberry and Cranberry Research and cultured on half-strength oatmeal agar. The medium was overlayed with cellulose acetate membranes to encourage sporulation (Stretch et al., 2001) and grown for 2 weeks under 8 h fluorescent light for production of conidia.

**Treatments:** There were four treatments; 1) untreated control, 2) pollinated only, 3) inoculated only, and 4) pollinated and inoculated. Eighty flowers/treatment/cultivar were used.

**Inoculation Procedure:** Flowers were tagged at anthesis. ‘Bluecrop’ pollen was applied to flowers 1 day after anthesis (treatments 2 and 4). Flowers were also inoculated as appropriate (treatments 3 and 4) with in vitro grown conidia of *M. vaccinii-corymbosi* on the same day. Untreated flowers at the same stage were also tagged.
Harvesting: Styles from each cultivar and each treatment were harvested 2 days post-treatment and used for RNA isolation. Ten flowers from each group were allowed to develop into fruit (if pollinated) in the greenhouse to verify successful treatment (fruit development and/or fungal colonization as appropriate).

RNA processing and transcriptome analysis: Total nucleic acid was isolated from the collected styles using the CTAB method as described (Polashock et al., 2009). RNA was precipitated from the total nucleic acid using lithium chloride. The transcriptome libraries were generated using the TruSeq RNA Sample Prep Kit (Illumina, San Diego, CA) and run on the Illumina HiSeqII platform. The resulting sequences were then quality- and adapter-trimmed with open-source software ‘cutadapt.’ Sequences were assembled using Velvet (Zerbino and Birney, 2008). Contigs were then BLASTed against the NCBI’s nucleotide database (blastn).

**Preliminary Results and Discussion**

We averaged 81 million reads and when assembled, produced 37,354 contigs for ‘Bluejay’ and 36,202 for ‘Berkeley’ with a length of at least 200 nucleotides. The sequences were then BLASTed against the NCBI’s nucleotide database (blastn) yielding 33,184,648 and 21,143,803 ‘hits’ for the two varieties, respectively. Many of the ‘hits’ were to similar genes from different plant species available in the database. Although we are still completing annotation and differential expression analyses, we have already identified several genes that are analogues to those associated with disease resistance in other species. We have detected surprisingly few fungal genes. One explanation is that the fungal RNA was underrepresented in the isolation since most of the tissue from which the RNA was isolated was from the plant (style).

**References**


